

# ADP Is a Competitive Inhibitor of ATP-Dependent H<sup>+</sup> Transport in Microsomal Membranes from *Zea mays* L. Coleoptiles

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## ABSTRACT

An anion-sensitive ATP-dependent H<sup>+</sup> transport in microsomal membranes from *Zea mays* L. coleoptiles was partially characterized using the pH gradient-dependent decrease of unprotonated neutral red. The following criteria strongly suggest a tonoplast origin of the H<sup>+</sup> transport observed: strict dependence on Cl<sup>−</sup>; inhibition by SO<sub>4</sub><sup>2−</sup> and NO<sub>3</sub><sup>−</sup>; insensitivity against vanadate, molybdate, and azide; reversible inhibition by CaCl<sub>2</sub> (H<sup>+</sup>/Ca<sup>2+</sup> antiport); inhibition by diethylstilbestrol. The substrate kinetics revealed simple Michaelis-Menten kinetics for ATP in the presence of 1 millimolar MgCl<sub>2</sub> with a *K<sub>m</sub>* value of 0.56 millimolar (0.38 millimolar for MgATP). AMP and c-AMP did not influence H<sup>+</sup> transport significantly. However, ADP was a potent competitive inhibitor with a *K<sub>i</sub>* value of 0.18 millimolar. The same inhibition type was found for membranes prepared from primary roots by the same procedure.

## MATERIALS AND METHODS

**Chemicals.** KS<sup>14</sup>CN (1.85 MBq·μmol<sup>−1</sup>) and [<sup>14</sup>C]methylamine (1.85 MBq·μmol<sup>−1</sup>) were from Amersham. Na<sub>2</sub>ATP and NaADP were vanadium free (Sigma). All other chemicals were of analytical grade.

**Plant Material.** Seeds of *Zea mays* L. (hybrid corn var IN-RAKORN category 2a, Deutsche Saatgut AG) were washed overnight in running tap water and then placed on moist filter paper and germinated in the dark at 23°C for 5 d. By that time, the coleoptiles had an average length of 4 cm. The coleoptiles were cut off in room light with a razor blade 2 mm from the node, and the primary leaves were removed. After collection on ice cold deionized H<sub>2</sub>O, they were filtered and blotted dry on filter paper. After determination of fresh weight, they were immediately immersed into the medium for plasmolysis (20 mM Mops<sup>1</sup> 400 mM sucrose, adjusted to pH 7.2 with 0.1 N KOH) for 30 min at 4°C. The tissue was degassed during the initial 10 min to facilitate the entry of the plasmolysis medium.

**Membrane Preparation.** The procedure followed the method of Hager and Helmle (14) with some modifications. The temperature throughout the preparation of membranes was kept at approximately 4°C. The tissue was extracted in a precooled mortar with 4 ml extraction buffer/g fresh weight: 100 mM Mops, 250 mM sucrose, 1 mM MgCl<sub>2</sub>, 10 mM EGTA, 20 mM ascorbic acid, 2.5 mM DL-DTT, 0.1% (w/v) BSA, adjusted to pH 7.2 with 1 N KOH. After slicing the tissue extensively with a razor blade, the extraction was completed by a short careful use of a pestle. The homogenate was passed through four layers of cheesecloth and the filtrate centrifuged for 30 min at 20,000g in a SS 34 rotor in a Sorvall centrifuge model RC-5B. The supernatant was centrifuged for 45 min at 50,000g in the same rotor. The pellet was resuspended in the same medium. Only in experiments with Ca<sup>2+</sup>, EGTA was omitted in the washing medium. After a second identical centrifugation step, the pellet was resuspended in 2 ml/g fresh weight in the same buffer including 50 mM KCl.

In some experiments, differential centrifugation was performed by collecting separately the 1,000g to 8,000g, 8,000g to 20,000g, and 20,000g to 50,000g pellets which were all washed and repelleted as described. After resuspension of the membranes, the pH was checked and corrected to 7.2.

**H<sup>+</sup> Transport Assay.** The membrane suspension was passed through a 20-μm nylon sieve and kept on ice until the assay period. The assay volume was 1 ml. The actual concentrations in the assay were 100 mM Mops, 250 mM sucrose, 1 mM MgCl<sub>2</sub>, 10 mM EGTA (omitted in the assays for Ca<sup>2+</sup> inhibition), 20 mM ascorbic acid, 2.5 mM DTT, 0.1% (w/v) BSA, 50 mM KCl, and

Recently, evidence has accumulated for the presence of two microsomal H<sup>+</sup>-ATPases in plants involved in the production of electrogenic H<sup>+</sup> gradients (4, 6, 8, 10, 11, 18, 19). One is associated with high density membranes (about 1.16–1.17 g/cm<sup>3</sup>) inhibited by vanadate and correlated with marker enzymes for plasma membrane (4, 7, 8, 19). The second one is found in low density membranes (about 1.10 g/cm<sup>3</sup>), activated by Cl<sup>−</sup>, inhibited by NO<sub>3</sub><sup>−</sup> and is thought to be of tonoplast origin (4, 9, 16, 17, 19). This latter assumption is strongly supported by recent results obtained with tonoplast vesicles prepared from previously isolated vacuoles (4) which show the same characteristics. Solubilization (20) and 'negative purification' (7) of both ATPases have been attempted which will help to further elucidate their reaction mechanism. Meanwhile, comparatively little efforts were undertaken to characterize the substrate kinetics (1, 9) of the tonoplast-type ATP-dependent H<sup>+</sup> transport although a kinetic regulation of the H<sup>+</sup> transport ATPases seemed likely (4).

In this study, we partially characterize a tonoplast-type ATP-dependent H<sup>+</sup> transport in microsomal membranes (4, 25). The disappearance of unprotonated neutral red in the reaction medium which reflects the accumulation of the protonated species in the membrane vesicles (14) is used to monitor H<sup>+</sup> transport. As free protonated neutral red is in equilibrium with membrane-bound protonated neutral red with different spectral characteristics (13), it is not opportune to follow the accumulation of the protonated species. We present evidence for a competitive inhibition of ATP-dependent H<sup>+</sup> transport by ADP under conditions where competition for ATP as substrate by ATPases other than the tonoplast-type ATPase is extensively reduced.

<sup>1</sup> Abbreviation: Mops, 3-(*N*-morpholino)propanesulfonic acid.

100  $\mu\text{M}$  neutral red, adjusted to pH 7.2 with 1 N KOH. Routinely membrane concentration was equivalent to 2 g fresh weight per ml assay medium. All substrate and inhibitor additions were made from concentrated stock solutions such as to account for less than 5% of the assay volume. Sodium vanadate solution was prepared according to Gallagher and Leonard (12).

The  $\Delta A$  at 430 nm was recorded in a Shimadzu double beam spectrophotometer model UV-150 with an identical reference cuvette where equimolar amounts of ADP were added instead of ATP. Reaction temperature was 25°C. The recorder was set to  $E = 0.1$  full deflection and 1  $\text{cm} \cdot \text{min}^{-1}$  chart speed. The initial rates of  $\text{H}^+$  transport were calculated from the slope between 1 and 4 min incubation which was linear in all assays.

**Accumulation of [ $^{14}\text{C}$ ]Methylamine and  $\text{S}^{14}\text{CN}^-$ .** For uptake of [ $^{14}\text{C}$ ]methylamine, the same assay medium and membrane concentration was used as described for the neutral red assay. [ $^{14}\text{C}$ ]Methylamine was added at a concentration of 2  $\mu\text{M}$  (1.85  $\text{MBq} \cdot \mu\text{mol}^{-1}$ ). The membrane suspension was stirred at 25°C and 0.5-ml aliquots were filtered at 2-min intervals over 0.45  $\mu\text{m}$  Sartorius membrane filters which were immediately rinsed with 5 ml [ $^{14}\text{C}$ ]methylamine free assay medium. One mM ATP or ADP were added after 4 min. For determination of radioactivity the filters were immersed into 3 ml of scintillant (Quickszint 212, Zinsser Analytic). The filters dissolved within 1 h. The samples were counted in a liquid scintillation counter Packard 3320. Their dpm values were calculated from a quench correction curve obtained by the channels ratio method. Counting times of individual samples were adjusted such as to accumulate at least 10,000 counts. The accumulation of  $\text{S}^{14}\text{CN}^-$  was determined in the absence of KCl.  $\text{KS}^{14}\text{CN}$  was added at 2  $\mu\text{M}$  (1.85  $\text{MBq} \cdot \mu\text{mol}^{-1}$ ). The filtration procedure and determination of radioactivity was as described for [ $^{14}\text{C}$ ]methylamine.

**Statistical Treatment of the Data.** All experiments were performed at least three times. Determinations within one experiment were done in duplicates. For all data except the substrate and competitive inhibition kinetics, the arithmetic means of the duplicates of one representative experiment are given. The  $K_m$  and  $K_i$  values were calculated from the Eadie-Hofstee plot,  $v = V_{\text{max}} - K_m \cdot v/C_s$ , by linear regression analysis and are presented  $\pm$  SD.

## RESULTS

**Characterization of the Anion-Sensitive ATP-Dependent  $\text{H}^+$  Transport.** A  $\text{Cl}^-$ -sensitive ATP-dependent  $\text{H}^+$ -transport was investigated in membrane fractions from 5-d-old maize coleoptiles and roots. After differential centrifugation, significant activity was found in the 1,000g to 8,000g, 8,000g to 20,000g, and

Table 1. Distribution of ATP-Dependent  $\text{H}^+$  Transport Activity in Different Membrane Fractions after Differential Centrifugation of Homogenates from 5-Day-Old Coleoptiles and Roots of Maize  
1,000g pellets and 50,000g supernatants were not included.

Plant Material	Coleoptiles	Roots
Total activity: Sum of all fractions (A-C) at a concentration of 1 g fresh wt $\cdot$ 4 $\text{ml}^{-1}$ final membrane suspension - $\Delta E_{430\text{nm}} \cdot \text{min}^{-1}$	0.056	0.032
Fraction A: 1,000g to 8,000g - $\Delta E_{430\text{nm}} \cdot \text{min}^{-1}$	0.022	0.018
% of total activity	39	54
Fraction B: 8,000g to 20,000g - $\Delta E_{430\text{nm}} \cdot \text{min}^{-1}$	0.022	0.010
% of total activity	39	31
Fraction C: 20,000g to 50,000g - $\Delta E_{430\text{nm}} \cdot \text{min}^{-1}$	0.012	0.005
% of total activity	22	15

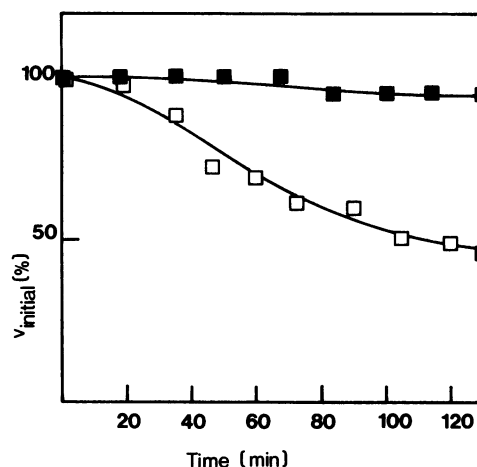


FIG. 1. Stability of  $\text{H}^+$  transport activity in the microsomal fraction (20,000g to 50,000g) of washed (■) or nonwashed (□) membranes from 5-d-old maize coleoptiles. 100% initial velocity equals an absorbance change  $\Delta E_{430\text{nm}} = -0.04 \cdot \text{min}^{-1}$ .

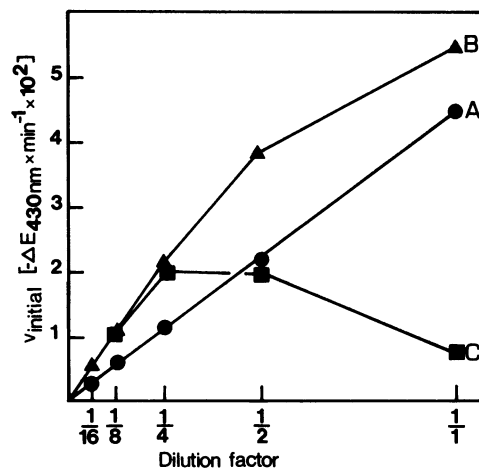


FIG. 2. Volume activity relationship of the  $\text{H}^+$  transport activity for different membrane fractions from 5-d-old maize coleoptiles. A, 20,000g to 50,000g; B, 8,000g to 20,000g; C, 1,000g to 8,000g. All fractions were washed and repelleted. Dilution factor 1/1 corresponds to 2 g fresh weight coleoptiles per ml final membrane suspension.

20,000g to 50,000g pellets (Table I). The stability of the ATP-dependent  $\text{H}^+$  transport was increased considerably by washing the membranes; otherwise, after 2 h, 60% of the initial activity was lost (Fig. 1). The volume activity relationship for the 1,000g to 8,000g pellet showed a severe inhibition at higher membrane concentrations (Fig. 2). The 8,000g to 20,000g fraction was inhibited to a lesser extent as membrane concentration was increased.

$\text{H}^+$  transport as measured by the decrease of unprotonated neutral red, which is the result of an accumulation of the protonated species in the membrane vesicles (13), was not influenced significantly by sodium vanadate, sodium molybdate, or sodium azide when KCl was present at 50 mM (Table II). Only in the 8,000g to 20,000g and the 20,000g to 50,000g pellets from root tissue did vanadate cause a slight inhibition of  $\text{H}^+$  transport.

The KCl-dependent acidification was strictly Mg-dependent and inhibited strongly by equimolar amounts of  $\text{K}_2\text{SO}_4$  and  $\text{KNO}_3$  (data not shown). DES at 100  $\mu\text{M}$  inhibited the  $\text{H}^+$  transport by about 80%. The comparison of the neutral red decrease with the accumulation of [ $^{14}\text{C}$ ]methylamine as determined with the filter assay showed almost identical kinetics (Fig. 3).

Table II. Effects of Vanadate, Molybdate, and Azide on the KCl-Stimulated ATP-Dependent H<sup>+</sup> Transport Activity (Decrease Unprotonated Neutral Red) in Different Membrane Fractions from 5-Day-Old Maize Coleoptiles and Primary Roots

Membrane Fraction	Initial H <sup>+</sup> Transport Rate		
	Sodium Vanadate (250 $\mu$ M)	Sodium Molybdate (1 mM)	Sodium Azide (1 mM)
% of control			
Coleoptiles			
1,000g to 8,000g	93	99	91
8,000g to 20,000g	86	95	85
20,000g to 50,000g	85	110	100
Primary roots			
1,000g to 8,000g	92	95	86
8,000g to 20,000g	76	107	90
20,000g to 50,000g	70	96	96

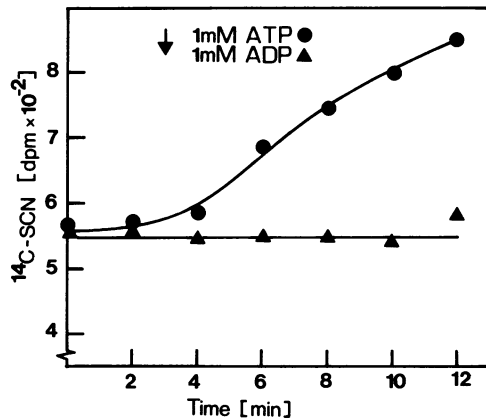


FIG. 3. Time course of development of ATP-dependent membrane potential in the microsomal fraction (20,000g to 50,000g) from 5-d-old maize coleoptiles. [<sup>14</sup>C]SCN<sup>-</sup> was added to washed and resuspended membranes in test medium without KCl. Aliquots were filtered through membrane filters (0.45  $\mu$ m) and the filters briefly washed with test medium without [<sup>14</sup>C]SCN<sup>-</sup>.

When KCl was omitted, an ATP-dependent accumulation of S<sup>14</sup>CN<sup>-</sup> was observed demonstrating the build up of a membrane potential inside positive (Fig. 4). Furthermore, the H<sup>+</sup> transport activity peaked at 1.1 g/cm<sup>3</sup> on a linear 10% to 45% sucrose gradient (data not shown).

All these results are in good agreement with well documented data on the H<sup>+</sup> transport driven by the tonoplast-type ATPase published elsewhere (4, 9, 16, 18, 26).

**H<sup>+</sup>/Ca<sup>2+</sup> Antiport in Membrane Vesicles.** The inhibition of the H<sup>+</sup> transport by relatively high CaCl<sub>2</sub> concentrations suggested the presence of a H<sup>+</sup>/Ca<sup>2+</sup> antiport. Membranes extracted in the presence of 10 mM EGTA and washed in medium without EGTA showed an inhibitory effect of Ca<sup>2+</sup> on the initial H<sup>+</sup> transport rate which was completely reversed by addition of EGTA (Figs. 3, 5, and 6). These data are in agreement with recent findings on the H<sup>+</sup>/Ca<sup>2+</sup> antiport in the tonoplast membrane (15, 21).

**Substrate Kinetics of the Anion-Sensitive ATP-Dependent H<sup>+</sup> Transport.** The following results were obtained with a washed membrane fraction (20,000g to 50,000g) in a reaction medium including routinely 250  $\mu$ M sodium vanadate, 1 mM sodium azide, and 50 mM KCl. Thus, any interference with other membrane bound ATPases competing for the substrate ATP was reduced. The high stability of the membranes allowed the determination of up to 20 assays of initial H<sup>+</sup> transport rates in one

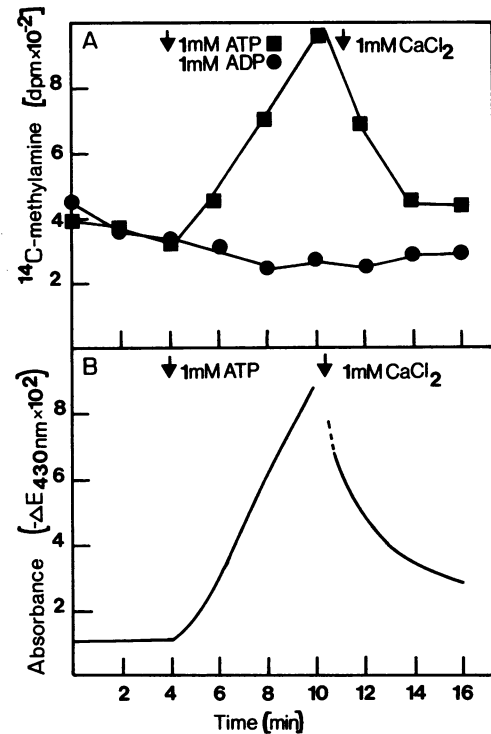


FIG. 4. Comparison of [<sup>14</sup>C]methylamine (A) and decrease of unprotonated neutral red (B) as indicators of ATP-dependent H<sup>+</sup> transport in the microsomal fraction (20,000g to 50,000g) from 5-d-old maize coleoptiles. A, 2  $\mu$ M [<sup>14</sup>C]methylamine was added to washed and resuspended membranes in test medium including 50 mM KCl. Determination of [<sup>14</sup>C]methylamine uptake as described for [<sup>14</sup>C]SCN<sup>-</sup> in Figure 3. B, 100  $\mu$ M neutral red was included in the test medium.

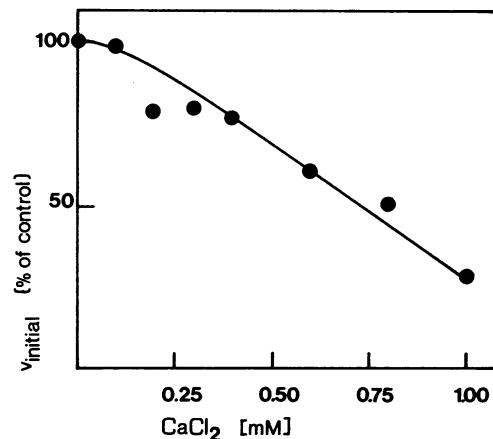


FIG. 5. Concentration dependence of the inhibitory effect of Ca<sup>2+</sup> on the H<sup>+</sup> transport activity in the microsomal fraction (20,000g to 50,000g) from 5-d-old maize coleoptiles. 100% initial velocity equals an absorbance change of  $\Delta E_{430\text{nm}} = -0.04 \cdot \text{min}^{-1}$ .

experiment. Comparison between different membrane batches was possible by adequate dilution of membranes such as to assure equal H<sup>+</sup> transport rates at 1 mM ATP.

The dependence of H<sup>+</sup> transport on the ATP concentration between 0.1 and 1.0 mM in the presence of 1 mM MgCl<sub>2</sub> showed a linear relationship in the Lineweaver-Burk plot (Fig. 7) for membranes both from coleoptiles and roots. For accurate determination of the  $K_m$  and the  $K_i$  values (see below), all data were transformed to the Eadie-Hofstee plot,  $v = V_{max} - K_m \cdot v/C_s$  (Figs. 8 and 9). For coleoptile, an apparent  $K_m$  value of  $0.56 \pm 0.08$

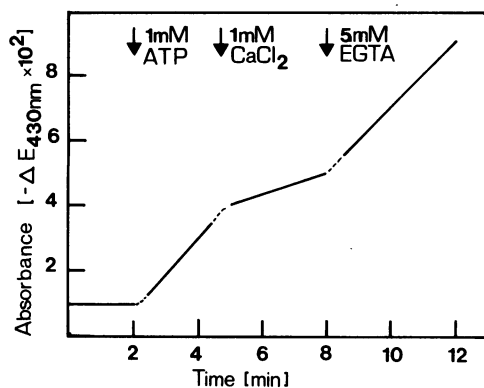


FIG. 6. Reversion of the inhibitory effect of  $\text{Ca}^{2+}$  on the  $\text{H}^+$  transport activity by EGTA. Microsomal fraction (20,000g to 50,000g) from 5-d-old maize coleoptiles. The slopes before addition of 1 mM  $\text{CaCl}_2$  and after addition of 5 mM EGTA are identical.

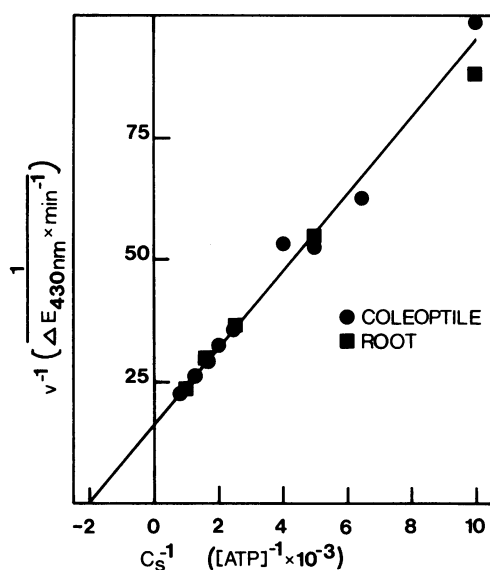


FIG. 7. Lineweaver-Burk plot of the ATP-dependent  $\text{H}^+$  transport in the microsomal fraction (20,000g to 50,000g) from 5-d-old maize coleoptiles and 5-d-old primary roots. The membrane suspensions of both tissues were diluted such as to give the same  $\text{H}^+$  transport rate at 1 mM ATP.

mm was obtained for ATP in the presence of 1 mM  $\text{MgCl}_2$ , the value for roots being  $0.52 \pm 0.07$  mM.

ADP significantly inhibited the  $\text{H}^+$  transport in membranes from both tissues. When the substrate kinetics for ATP between 0.4 and 1.0 mM were determined in the presence of different ADP concentrations (0.2, 0.4, and 0.6 mM), the results indicated a competitive inhibition for membranes from coleoptiles (Fig. 8). The  $K_i$  value was calculated from the intercepts with the horizontal axis which are  $V_{\max}/K_m(1 + C_i/K_i)$ . Resolution for  $K_i$  according to  $K_i = K_m C_i / (V_{\max}/X_{\text{intercept}} - K_m)$  gave a value of  $0.18 \pm 0.03$  mM.

Although the results with membranes from primary roots somewhat deviated from ideal competitive inhibition kinetics, this type of inhibition is still highly probably for roots as well (Fig. 9). AMP and c-AMP did not cause any significant inhibition of  $\text{H}^+$  transport at concentrations up to 1 mM (data not shown).

## DISCUSSION

Several lines of evidence strongly support the assumption that with the method chosen for this study, which is a modification

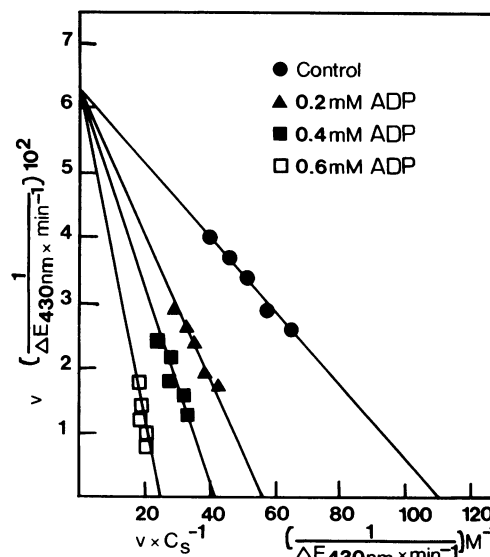


FIG. 8. Eadie-Hofstee plots of the ATP-dependent  $\text{H}^+$  transport in the microsomal fraction (20,000g to 50,000g) from 5-d-old maize coleoptiles in the presence of different ADP concentrations. The  $K_m$  value for ATP in the presence of 1 mM  $\text{MgCl}_2$  was  $0.56 \pm 0.08$  mM. The  $K_i$  value obtained for ADP was  $0.18 \pm 0.03$  mM.

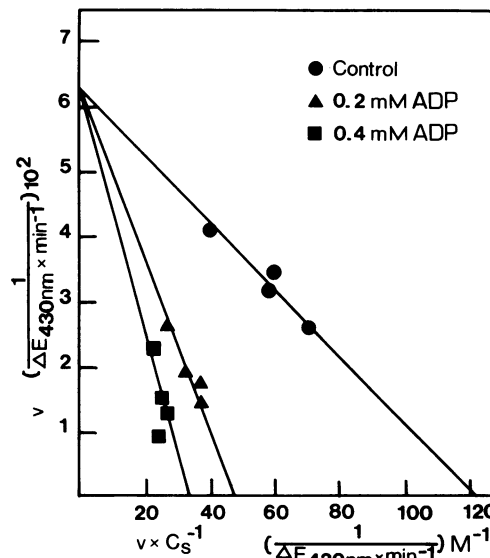


FIG. 9. Eadie-Hofstee plots of the ATP-dependent  $\text{H}^+$  transport in the microsomal fraction (20,000g to 50,000g) from 5-d-old primary roots of maize. The  $K_m$  value for ATP in the presence of 1 mM  $\text{MgCl}_2$  was  $0.52 \pm 0.07$  mM. The  $K_i$  value obtained for ADP was  $0.14 \pm 0.03$  mM.

of the procedure of Hager and Helmle (14), the ATP-dependent  $\text{H}^+$  transport into membrane vesicles, possibly of tonoplast origin, may be accurately determined. The following results support the view that, by monitoring the decrease of unprotonated neutral red (13), we indirectly followed the ATP-dependent  $\text{H}^+$  transport driven by the tonoplast-type ATPase: (a) the dependence on  $\text{Mg}^{2+}$  ions; (b) the identical kinetics of the ATP-dependent decrease of unprotonated neutral red and the accumulation of  $[^{14}\text{C}]$ methylamine (9, 16; Fig. 3); (c) the strong stimulation by 50 mM KCl (4, 20; Martina Ziemann-Roth, unpublished results); (d) the reversal of  $\text{Cl}^-$  stimulation by equimolar amounts of  $\text{SO}_4^{2-}$  and  $\text{NO}_3^-$  (14, 18); (e) the absence of any significant effects of vanadate, molybdate, and azide, which inhibit the plasma membrane  $\text{H}^+$ -ATPase (4), the acid phosphatase

tase (12), and the mitochondrial ATPase (12), respectively (Table II); (f) the strong inhibition by DES (4); (g) the reversible inhibition by CaCl<sub>2</sub>, supporting the presence of a H<sup>+</sup>/Ca<sup>2+</sup> antiport (15, 21; Figs. 3, 5, and 6).

When vanadate and azide were included at concentrations reducing the competition of plasma membrane and mitochondrial ATPases for ATP as substrate, the initial rate of H<sup>+</sup> transport should give a correct estimate of the transport activity of the tonoplast-type H<sup>+</sup>-ATPase. Additional criteria like a sufficient stability of the transport activity and a linear volume activity relationship were equally met and allowed a correct analysis of the kinetic data (Figs. 1 and 2). Thus, it was possible to study the substrate kinetics and the inhibition by ADP of the tonoplast-type ATP driven H<sup>+</sup> transport in a membrane fraction (20,000g to 50,000g) obtained by simple differential centrifugation circumventing the time consuming use of an ultracentrifuge.

The apparent  $K_m$  values obtained for the ATP-dependent H<sup>+</sup> transport of membrane vesicles both from primary roots and coleoptiles are almost identical (Fig. 7) and in good agreement with recent data obtained with an ATPase from vacuoles and tonoplast vesicles isolated from *Kalanchoë daigremontiana* (1). The lower  $K_m$  value of 0.11 mM published by Churchill and Sze (9) for the ATP-dependent uptake of [<sup>14</sup>C]methylamine into membrane vesicles of oat root may be due to experimental differences or to species variation. Furthermore, the presentation of the data suggests that the authors used the concentration of the Mg-ATP complex which may be regarded as the proper substrate rather than ATP concentrations. Our data when calculated on the basis of Mg-ATP concentrations (27) give a  $K_m$  value of 0.38 mM. From a comparison of the available data, it may be concluded that the initial H<sup>+</sup> transport rate is directly proportional to the amount of ATP hydrolyzed. This assumption is further supported by a recent study on the H<sup>+</sup>/ATP stoichiometry (6; see below).

The addition of ADP resulted in a competitive inhibition of the H<sup>+</sup> transport with a rather low  $K_i$  value of 0.18 mM (Figs. 8 and 9), while AMP showed no effect. Thus, the *in vivo* activity of the tonoplast-type ATPase seems to be under the control of the cytoplasmic ADP/ATP ratio. Assuming an intracellular ATP concentration of about 1 mM and an ADP/ATP ratio of 0.3 (6), it may be calculated that the tonoplast-type ATPase *in vivo* shows only about 40% of its maximum activity:

$$1/v = K_m/V_{max}(1/ATP)(1 + ADP/K_i) + 1/V_{max}$$

when ATP = 1 mM, ADP = 0.3 mM,  $K_m$  = 0.56 mM,  $K_i$  = 0.18 mM, and  $V_{max}$  = 0.063  $\Delta E_{430\text{ nm}} \cdot \text{min}^{-1}$ , it follows that

$$v = 0.025 \Delta E_{430\text{ nm}} \cdot \text{min}^{-1} \approx 40\% \text{ of } V_{max}$$

*In vivo*, the intracellular ADP/ATP ratio strictly regulates the H<sup>+</sup> transport into the vacuole assuring a physiologically highly significant control mechanism for the intracellular ATP consumption. In a recent study on the H<sup>+</sup>/ATP stoichiometry of an anion sensitive H<sup>+</sup>-ATPase from storage tissue of *Beta vulgaris*, Bennett and Spanswick (6) obtained a value of 2 H<sup>+</sup> per ATP hydrolyzed. However, from a comparison with *in vivo* data on  $\Delta$ pH and  $\Delta$ Ψ across the tonoplast membrane (2, 22–24) the authors conclude that, *in vivo*, the theoretically possible values are rarely found and assume a kinetic regulation by cellular factors. We propose that the competitive inhibition of the H<sup>+</sup> transport by ADP is one of these signals.

After completion of this manuscript, Bennett *et al.* (3) pub-

lished results indicating that, in red beet, both plasma membrane and the tonoplast ATPases are competitively inhibited by ADP.

#### LITERATURE CITED

1. AOKI K, K NISHIDA 1984 ATPase activity associated with vacuoles and tonoplast vesicles isolated from the CAM plant, *Kalanchoë daigremontiana*. *Physiol Plant* 60: 21–25
2. BATES GW, MHM GOLDSMITH, TH GOLDSMITH 1982 Separation of tonoplast and plasma membrane potential and resistance in cells of oat coleoptiles. *J Membr Biol* 66: 15–23
3. BENNETT AB, SD O'NEILL, M EILMANN, RM SPANSWICK 1984 Modulation of H<sup>+</sup>-ATPase by reaction substrates and products. *Plant Physiol* 75: S-42
4. BENNETT AB, SD O'NEILL, RM SPANSWICK 1984 H<sup>+</sup>-ATPase activity from storage tissue of *Beta vulgaris*. I. Identification and characterization of an anion-sensitive H<sup>+</sup>-ATPase. *Plant Physiol* 74: 538–544
5. BENNETT AB, RM SPANSWICK 1983 Optical measurements of  $\Delta$  pH and  $\Delta$  Ψ in corn root membrane vesicles. Kinetic analysis of Cl<sup>−</sup> effects on a proton-translocating ATPase. *J Membr Biol* 71: 95–107
6. BENNETT AB, RM SPANSWICK 1984 H<sup>+</sup>-ATPase activity from storage tissue of *Beta vulgaris*. II. H<sup>+</sup>/ATP stoichiometry of an anion-sensitive H<sup>+</sup>/ATP stoichiometry of an anion-sensitive H<sup>+</sup>-ATPase. *Plant Physiol* 74: 545–548
7. BRISKIN DP, RT LEONARD 1982 Phosphorylation of the adenosine triphosphatase in a deoxycholate-treated plasma membrane fraction from corn roots. *Plant Physiol* 70: 1459–1464
8. BRISKIN DP, RJ POOLE 1983 Characterization of a K<sup>+</sup>-stimulated ATPase associated with the plasma membrane of red beet. *Plant Physiol* 71: 350–355
9. CHURCHILL KA, H SZE 1983 Anion-sensitive H<sup>+</sup>-pumping ATPase in membrane vesicles from oat roots. *Plant Physiol* 71: 610–617
10. DUPONT FM, AB BENNETT, RM SPANSWICK 1982 Localization of proton-translocating ATPase on sucrose gradients. *Plant Physiol* 70: 1115–1119
11. DUPONT FM, DL GIORGI, RM SPANSWICK 1982 Characterization of a proton-translocating ATPase in microsomal vesicles from corn roots. *Plant Physiol* 70: 1694–1699
12. GALLAGHER SR, RT LEONARD 1982 Effect of vanadate, molybdate, and azide on membrane-associated ATPase and soluble phosphatase activities of corn roots. *Plant Physiol* 70: 1335–1340
13. HAGER A, R FRENZEL, D LAIBLE 1980 ATP-dependent proton transport into vesicles of microsomal membranes of *Zea mays* coleoptiles. *Z Naturforsch* 35c: 783–793
14. HAGER A, M HELMLE 1981 Properties of an ATP-fueled, Cl<sup>−</sup> dependent proton pump localized in membranes of microsomal vesicles from maize coleoptiles. *Z Naturforsch* 36c: 997–1008
15. HAGER A, P HERMSDORF 1981 A H<sup>+</sup>/Ca<sup>2+</sup> antiporter in membranes of microsomal vesicles from maize coleoptiles, a secondary energized Ca<sup>2+</sup> pump. *Z Naturforsch* 36c: 1009–1012
16. MANDALA S, IJ METTLER, L TAIZ 1982 Localization of the proton pump of corn coleoptile microsomal membranes by density gradient centrifugation. *Plant Physiol* 70: 1743–1747
17. METTLER IJ, S MANDALA, L TAIZ 1982 Characterization of *in vitro* proton pumping by microsomal vesicles isolated from corn coleoptiles. *Plant Physiol* 70: 1738–1742
18. O'NEILL SD, AB BENNETT, RM SPANSWICK 1983 Characterization of a NO<sub>3</sub><sup>−</sup>-sensitive H<sup>+</sup>-ATPase from corn roots. *Plant Physiol* 72: 837–846
19. POOLE RJ, DP BRISKIN, Z KRATKY, RM JOHNSTONE 1984 Density gradient localization of plasma membrane and tonoplast from storage tissue of growing and dormant red beet. Characterization of proton-transport and ATPase in tonoplast vesicles. *Plant Physiol* 74: 549–556
20. RANDALL SK, Y WANG, H SZE 1984 Solubilization and characterization of an anion-sensitive H<sup>+</sup>-ATPase from oat roots. *Plant Physiol* 75: S-2
21. RASI-CALDOGNO F, MI DE MICHAELIS, MC PUGLIARELLO 1982 Active transport of Ca<sup>2+</sup> in membrane vesicles from pea. Evidence for a H<sup>+</sup>/Ca<sup>2+</sup> antiport. *Biochim Biophys Acta* 693: 287–295
22. ROBERTS JKM, PM RAY, N WADE-JARDETSKY 1980 Estimation of cytoplasmic and vacuolar pH in higher plant cells by <sup>31</sup>P-NMR. *Nature (Lond)* 283: 870–872
23. SMITH FA, JA RAVEN 1979 Intracellular pH and its regulation. *Annu Rev Plant Physiol* 30: 289–311
24. SPANSWICK RM, EJ WILLIAMS 1964 Electrical potentials and Na, K, and Cl concentrations in the vacuole and cytoplasm of *Nitella translucens*. *J Exp Bot* 15: 422–427
25. WAGNER GJ, P MULREADY 1983 Characterization and solubilization of nucleotide-specific Mg<sup>2+</sup>-ATPase and Mg<sup>2+</sup>-pyrophosphatase of tonoplast. *Biochim Biophys Acta* 728: 267–280
26. WALKER RP, RA LEIGH 1981 Characterization of a salt stimulated ATPase activity associated with vacuoles isolated from storage roots of red beet (*Beta vulgaris* L.). *Planta* 153: 140–149
27. WOLF HU, L ADOLPH 1969 Untersuchungen über eine Mg<sup>2+</sup>-(Ca<sup>2+</sup>)-aktivierte ATPase aus Rinderhirnmikrosomen. *Eur J Biochem* 8: 68–74